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On: (1) BIOLOGICAL EFFECTS OF PROLONGED EXPOSURE OF SMALL ANIMALS TO UNUSUAL GAS ENVIRONMENTS ★

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BIOLOGICAL EFFECTS OF PROLONGED EXPOSURE OF
SMALL ANIMALS TO UNUSUAL GAS ENVIRONMENTS

This is the third semi-annual progress report and again our results are best discussed under several headings.

1. Exposure of mice to pure oxygen at one atmosphere with varying daily periods of air breathing

There is evidence in the literature that the toxic effects of oxygen at high partial pressures can be reduced by relatively short periods of exposure to air (for example, see Penrod, J. Appl. Physiol. 9: 149, 1956). To examine this important hypothesis we set up two 20-day experiments, the results of which can be summarized together.

Arrangements were made to keep cages, each containing four purebred adult male white mice in a chamber containing almost pure oxygen at one atmosphere of pressure. Temperature and humidity were regulated and CO_2 absorbed by soda lime. Oxygen was admitted via an O_2 mask regulator so that the system was about two cm of water above atmosphere pressure. There was some leakage so that the system was not completely closed. A lock was attached to the chamber which could be flushed with oxygen so that animals could be moved from the chamber to the outside and vice versa without contaminating the chamber with air. The animals were handled within the chamber by means of long rubber gloves attached to ports in the wall. The oxygen concentration was determined several times daily and maintained at more than 98%.

The period of exposure, the number of animals, and the median time of death are tabulated below.

Group	No. animals	Daily period in oxygen	Median time to death
1	16	0 (air controls)	all survived 20 days
2	8	8 hrs	all survived 20 days
3	16	16 hrs	all survived 20 days
4	8	20 hrs	10 days - 3 survived 20 days
5	8	22 hrs	8 days - 1 survived 20 days
6	16	24 hrs	5 days - none survived after 7 days

The median time to death is the length of time required for half of the animals in a group to die. We realize that this may not be the best method of expressing the results of toxicity testing but it suits our present purpose best, particularly since some of the animals survived the test period in all groups except the last.

In addition to observing survival we obtained body weights and measured water consumption. A decrease in water consumption is a convenient and sensitive index of the health of these animals as indicated in Fig. 1. These curves represent a visual best-fit of the data points and consequently do not show the daily variation. It is apparent that animals exposed at least 16 hours a day to pure oxygen show marked depression of their water intake within 24 hours.

We also sacrificed some animals for study of the histology of the lungs in the 8- and 16-hour per day oxygen exposure groups at the end of the 20-day exposure period.

Four animals were sacrificed from each group, two at the end of their daily period of oxygen exposure while still in oxygen, and two after their daily period in air. In addition we sacrificed two control animals and four animals exposed to 24-hr-per-day oxygen, one each after 24, 48, 72, and 96 hours of exposure.

Dr. John Durfey, a former member of our group, has observed that there were marked changes in the lungs of dead mice which were allowed to stay in one atmosphere of oxygen for a period of hours before autopsy. (He will report his findings at the Aerospace Medical Meetings in April, 1964.) Consequently we did not do any histology on mice found dead but sacrificed living animals by breaking their necks. Then their tracheas were tied off to keep the lungs from collapsing and the lungs quickly removed and put into the fixative solution. The lungs were weighted so that they would be immersed. After fixing and embedding in paraffin they were sectioned horizontally at routine thickness and stained with hematoxylin and eosin. The sections were examined by Dr. Phillip Pratt of The Ohio State University Department of Pathology. Dr. Pratt is an authority on the pulmonary pathology of oxygen toxicity. (Pratt, P. C., "Pulmonary capillary proliferation induced by oxygen inhalation," Am. J. Pathol. 34: 1033, 1958; also article in press in Annals of N. Y. Acad. Science).

The animals sacrificed after exposure to one atmosphere of oxygen for periods up to 96 hours showed gradually increasing lung damage, beginning at 24 hours, with scattered alveolar collapse and congestion and progressing to septal thickening, edema, and hemorrhage by 96 hours.

The animals exposed for 20 days to the oxygen atmosphere for eight hours per day showed some alveolar collapse and congestion which was greater in those sacrificed after the period of oxygen exposure than in those sacrificed after the period in air.

The animals exposed for 20 days to the oxygen atmosphere for 16 hours per day showed a greater degree of damage than those exposed to eight hours per day with some hemorrhage. However in this group also the changes were more marked in the animals sacrificed after their period of oxygen exposure than in those sacrificed after their period of air exposure.

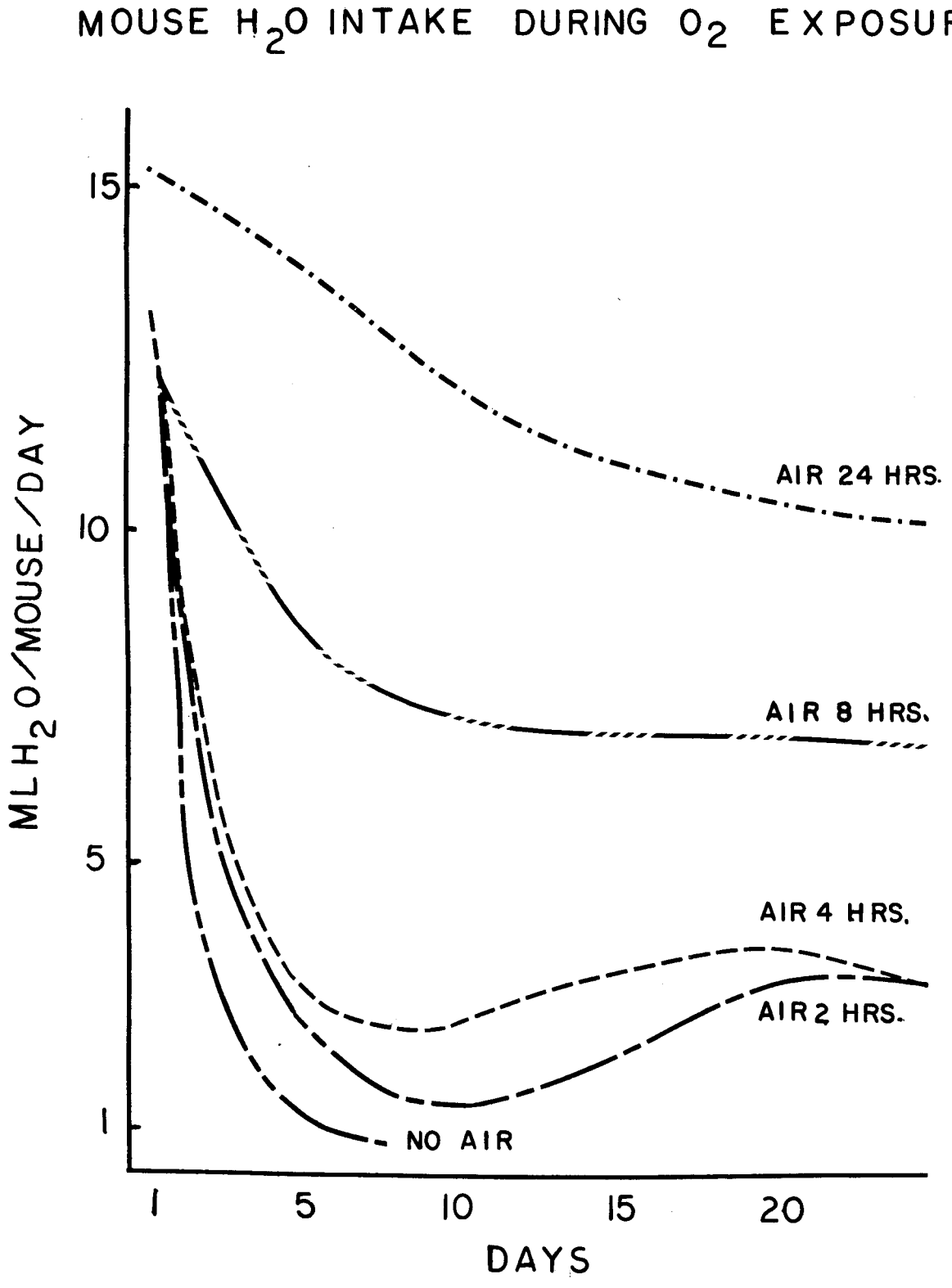
MOUSE H_2O INTAKE DURING O_2 EXPOSURE
ML H_2O/MOUSE/DAY
15
10
5
1
AIR 24 HRS.
AIR 8 HRS.
AIR 4 HRS.
AIR 2 HRS.
NO AIR
1
5
10
15
20
DAYS

Fig. 1

To explore the possibility of adaptation, suggested by the survival of some animals in the groups exposed to oxygen for 16, 20, and 22 hours, we conducted one more experiment in this series. Eight of the survivors in these groups were kept continuously in the oxygen atmosphere along with a group of control animals which had not previously been exposed to such oxygen atmospheres. The median lethal time for the control animals was four days while that for the previously exposed animals was seven days. Whether this difference is due to an adaptive process or to selection of resistant animals is not clear in this preliminary experiment, but it is certain that the previous 20-day exposure period did not make the animals more vulnerable in the continuous exposure situation.

In summary it appears that:

(1) Mice can survive exposure to one stmosphere of oxygen better if they have a daily period of air breathing as short as two hours and that the benefit increases with the time of air exposure per day up to at least eight hours.

(2) The lungs of mice show pathological changes when the mice are exposed to one atmosphere of oxygen for periods of at least eight hours a day. The severity of the damage increases with the daily time of exposure to oxygen but reverses rapidly on exposure to air.

(3) Animals which have survived a 20-day exposure to one atmosphere of oxygen, with daily periods of air breathing lasting from 2 to 16 hours per day, are not more vulnerable to the effects of continuous exposure to their environment than are previously unexposed mice. (We plan to present a report on this work at the Fall meetings of the Physiological Society.)

a. General significance and future plans

These studies apply to any situation in which animals are exposed to enriched oxygen atmospheres. The possibility that intermittent dilution of the oxygen by an inert gas can increase the resistance to oxygen toxicity is of great importance, not only in the closed environments of space capsules but in the therapeutic and aviation applications of enriched oxygen environments. Previous experimentation in this and other laboratories would indicate that the benefit is due to the reduced P_{O_2} rather than the addition of nitrogen. (Dines, J. and E. Hiatt, J. Appl. Physiol. 19: 17, 1964; MacHattie, L. and H. Rahn, Proc. Soc. Exp. Biol. Med. 104: 772, 1960).

Dr. Pratt believes that the mouse is not as satisfactory for histological study as other animals such as rats, dogs, or monkeys, because the latter show changes more like those seen in man. Control mice show considerable variability perhaps because of intercurrent respiratory infection.

Consequently we shall probably continue our studies with these other animals. We are considering other programs of intermittent exposure and lower pressures of oxygen as well as variations in the inert gas component.

2. The influence of prolonged exposure of chickens to almost pure oxygen at one atmosphere of pressure

In our last report we mentioned that when we put chickens and mice in oxygen at one atmosphere the chickens continued to live in apparent health for weeks after all the mice had died. This was verified in another experiment utilizing very young chicks with control animals in an identical chamber breathing air. This time we measured weight gain and food consumption and found that, although the chicks in oxygen continued to live and grow, they did not grow as fast as the controls in air. Other measurements made on the chicks included body temperature, blood hemoglobin, red cell count, hematorcrit and blood pH which did not change. However, the heart rate and respiratory rate of the chicks in oxygen decreased as compared with the controls.

After four weeks the conditions in the two chambers were reversed so that the former air breathing controls now breathed pure oxygen, and the former O_2 breathing chicks now were in air. A reversal of the trend of the growth curves occurred, perceptible within the first two days (Fig. 2). Evidently, although chickens seem to survive one atmosphere of oxygen much better than mammals, they suffer some depressive metabolic influence. Whatever this is, it comes on quickly and is reversed quickly on return to air breathing.

This work will be reported by Dr. Weiss at the meetings of the Federation of American Societies for Experimental Biology in Chicago, April, 1964.

3. Tissue metabolism of chicken embryos incubated in an atmosphere of 79% helium plus 21% oxygen

Dr. Ronald Wright, an NASA trainee, is continuing his studies of the effect of a nitrogen-free atmosphere on the tissue metabolism of chick embryos. The nitrogen-free atmosphere in this series of experiments was achieved by incubating eggs in a mixture of 79% helium and 21% oxygen.

In an initial series he used intact four-day embryos measuring their oxygen consumption and the effect of 250 r of X-irradiation. He found little difference between the metabolism of the He + O_2-incubated eggs and their controls incubated in air and little effect on the radiation. There was considerable variation in embryos, perhaps reflecting their different ages at the beginning of the incubation period.

In a second series he used homogenates of eight-day embryos which gave a more consistent preparation. He also increased the radiation dosage to 600 r and 1200 r. In this series the eggs incubated in helium and oxygen

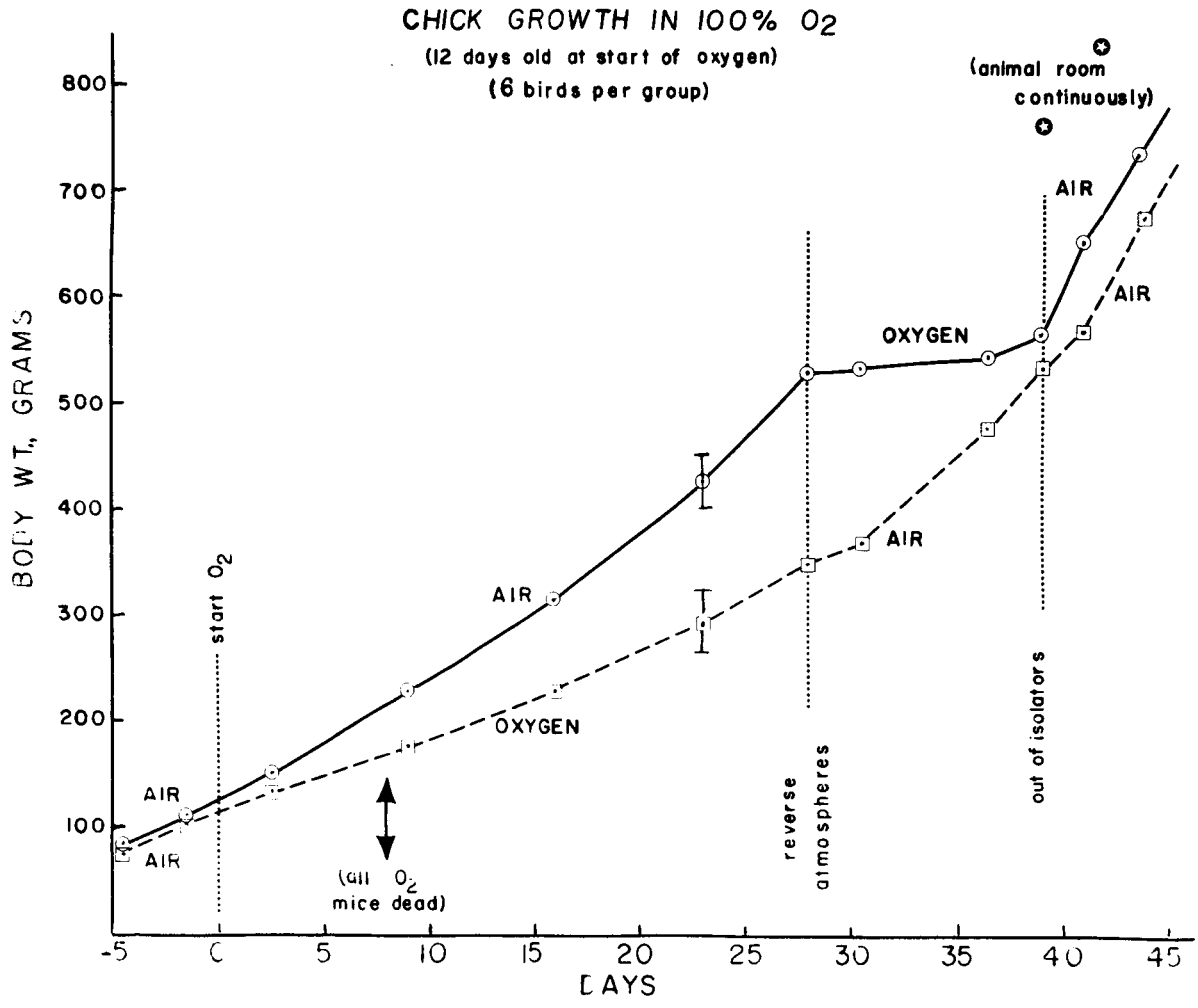
CHICK GROWTH IN 100% O2
(12 days old at start of oxygen)
(6 birds per group)
(animal room continuously)
BODY WT., GRAMS
800
700
600
500
400
300
200
100
start O2
AIR
OXYGEN
AIR
AIR
AIR
OXYGEN
(all O2 mice dead)
reverse atmospheres
out of isolators
-5
0
5
10
15
20
25
30
35
40
45
DAYS

Fig. 2

show a reduced rate of oxygen consumption. The metabolism was depressed in all preparations as a result of the irradiation but less so in those incubated in helium and oxygen.

These studies are continuing with some specific studies of enzymes. We also have acquired some large flasks to use with the Warburg technique to measure oxidative metabolism in intact eggs and small mammals. With this approach we hope to obtain serial observations on the same subject.

4. Development of the avian embryo in an atmosphere almost devoid of nitrogen

a. Pure oxygen at a reduced total pressure

Prior to the interruption of this specific area of work by a fire in our altitude chamber (noted in our last semi-annual report) we had found that the chick embryos developing in 100% O_2 at about 200 mm Hg were always smaller, even after only four days of incubation. We had not been able to pursue these experiments to the point where we could tell whether the reduced embryonic growth was a function of hypoxia, lack of inert gas, low pressure, or, since the chamber required a constant O_2 flush, possibly to excess moisture loss. While waiting for the repair of the fire-damaged chamber, we have continued these studies in a simplified, miniaturized, low-pressure system in which the experimental chamber is a series of plastic cylinders about 3 inches in diameter and 18 inches long, closed at the ends by rubber stoppers. Each cylinder, which can hold about six eggs (or a rat or several mice), is connected to its own circulating pump, CO_2 absorber, O_2 reservoir, temperature probe and pressure gage, and can be filled with 100% O_2 to any pressure between atmospheric and 100 mm Hg. The entire system is in turn placed in a temperature-controlled box.

So far we have found these systems can be charged with sufficient O_2 at the start of a test to support embryonic growth for eight days, even at reduced pressure, and that it is possible to keep them completely sealed for such periods of time with no loss in pressure or contamination with N_2.

In two eight day trials with fertile eggs, using 100% O_2 at total pressures ranging from 175 to 250 mm Hg, we have found no difference in the appearance or weights of embryos in comparison to similarly treated air controls. Tentatively, then, we believe that our early results of depressed growth in the altitude chamber was more a function of the constant O_2 flush than the pressure and composition of the atmosphere. It is possilbe that with a few modifications we may be able to carry these embryos for 21 days, or until they hatch, and thus be able to determine whether the N_2 deficiency caused effects not detectable at eight days by gross examination or by weight changes.

(b) 79% He - 21% O_2 at one atmosphere

One of the possibilities which should be considered in trying to explain the large number of abnormal embryos and poorer hatchability in N_2-low atmospheres is interruption of the normal fixation of gaseous N_2 into tissue N_2 by the avian embryo. (Volskii, M. I., Doklady Akademii Nauk S.S.S.R., 28: 895, 1960, Translation). In an N_2-low or N_2-free environment, N_2 fixation would, presumably be reduced or stopped. To test this hypothesis, we measured the total N_2 of fertile eggs incubated for up to 18 days in air and in He-O_2. The intact egg, shell and embryo, was digested first in concentrated HCl, then throughly mixed and aliquots removed for macro-Kjeldahl determination of N_2. Nitrogen was expressed in terms of the weights of the fresh eggs as measured at the start of incubation. We have found no difference in the N_2 content of the eggs incubated in the two atmospheres. While it is true that the He-O_2 atmosphere may have contained up to 2% N_2, this atmosphere is still typical of the kind which resulted in abnormal embryos and poor hatches. We tentatively conclude that some explanation other than a failure of N_2 fixation must to be sought for the effects of N_2-low atmospheres on the avian embryo.

Investigator ..... Harold S. Weiss ..... Date March 17, 1964

Supervisor ..... S. P. Lall ..... Date Mar. 18, 1964

For The Ohio State University Research Foundation

Executive Director ..... Robert C. Stephenson ..... Date 3/19/64